

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION
(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
 United States Patent and Trademark
 Office
 Box PCT
 Washington, D.C.20231
 ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 19 November 1999 (19.11.99)
International application No. PCT/GB99/00876
International filing date (day/month/year) 19 March 1999 (19.03.99)

Applicant's or agent's file reference
SP/CT/N7947

Priority date (day/month/year)
19 March 1998 (19.03.98)

Applicant

EBRINGER, Alan

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

14 October 1999 (14.10.99)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Olivia RANAIVOJAONA
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference SP/CT/N7947	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 99/ 00876	International filing date (day/month/year) 19/03/1999	(Earliest) Priority Date (day/month/year) 19/03/1998
Applicant KING'S COLLEGE, UNIVERSITY OF LONDON et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

contained in the international application in written form.

filed together with the international application in computer readable form.

furnished subsequently to this Authority in written form.

furnished subsequently to this Authority in computer readable form.

the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. **Certain claims were found unsearchable** (See Box I).

3. **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

the text is approved as submitted by the applicant.

the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

the text is approved as submitted by the applicant.

the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

as suggested by the applicant.

because the applicant failed to suggest a figure.

because this figure better characterizes the invention.

None of the figures.

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>B. H. TOH ET AL.: "The 200- and 150-kDa neurofilament proteins react with IgG autoantibodies from patients with kuru, Creutzfeldt-Jakob disease, and other neurologic diseases."</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 82, May 1985 (1985-05), pages 3485-3489, XP002052986 WASHINGTON US</p> <p>---</p>	
A	<p>R. L. SIDMAN ET AL.: "Transmissible spongiform encephalopathy in the gray tremor mutant mouse."</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 82, January 1985 (1985-01), pages 253-257, XP002052987 WASHINGTON US</p> <p>---</p>	
A	<p>CHEMICAL ABSTRACTS, vol. 109, no. 21, 21 November 1988 (1988-11-21) Columbus, Ohio, US; abstract no. 187890,</p> <p>M. P. MCKINLEY ET AL.: "Developmental regulation of prion protein mRNA in brain."</p> <p>page 484; column 2; XP002052989 abstract</p> <p>& CIBA FOUND. SYMP., vol. 135(Novel Infect. Agents Cent. Nerv. Syst.), 1988, pages 101-116,</p> <p>---</p>	
P, X	<p>WO 98 13694 A (KING'S COLLEGE) 2 April 1998 (1998-04-02) the whole document</p> <p>---</p>	1-13

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/00876

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CHEMICAL ABSTRACTS, vol. 80, no. 11, 18 March 1974 (1974-03-18) Columbus, Ohio, US; abstract no. 56313, A. WAJGT.: "Assessment by immunofluorescence methods of humoral antimyelin antibody in rats with cyanide encephalopathy." page 68; column 1; XP002052988 abstract & ANN. IMMUNOL. (POZNAN), vol. 5, no. 1-2, 1973, pages 51-58, ---</p> <p style="text-align: center;">-/-</p>	1

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

22 September 1999

Date of mailing of the international search report

30/09/1999

Name and mailing address of the ISA

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Authorized officer

Griffith, G

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/00876

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9813694 A	02-04-1998	EP 0929813 A	21-07-1999

PATENT COOPERATION TREATY

PCT

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RECD 19 JUL 2000
WIPO
PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference SP/GM/N7947	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB99/00876	International filing date (day/month/year) 19/03/1999	Priority date (day/month/year) 19/03/1998
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant KING'S COLLEGE, UNIVERSITY OF LONDON et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 14/10/1999	Date of completion of this report 14.07.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Renggli, J Telephone No. +49 89 2399 7461



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/00876

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-5 as originally filed

Claims, No.:

8-15 with telefax of 14/02/2000

1-7 with telefax of 03/07/2000

Drawings, sheets:

1/2,2/2 as originally filed

2. The amendments have resulted in the cancellation of:

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

3. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

II. Priority

1. This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

copy of the earlier application whose priority has been claimed.
 translation of the earlier application whose priority has been claimed.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/00876

2. This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-15
	No:	Claims
Inventive step (IS)	Yes:	Claims 1-15
	No:	Claims
Industrial applicability (IA)	Yes:	Claims 1-15
	No:	Claims

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/00876

ITEM I: Additional observations

Sequence listing page 1 filed with the letter of 23.7.99 does not form part of the application (Rule 13ter.1(f) PCT).

ITEM II: Additional observations

The present application claims priority from GB 9805913.2 filed on March 19th 1998 (Art. 8 PCT).

Claims 1-10, 13 and 15 of the present application have a valid claim to priority from GB 9805913.2. The relevant date for the said claims is therefore March 19th 1998. D1 (WO 98/13694) published on 2.4.1998 is therefore not relevant prior art within the meaning of Article 33 and Rule 64.1 PCT for the said claims.

Claims 11, 12 and 14 of the present application do not have a valid claim to priority from GB 9805913.2, since this latter document does not disclose the peptide sequences ISRFAWGEV or RFSAWGAE. The subject-matter of claims 11, 12 and 14 is therefore not directly and unambiguously derivable from the disclosure of the invention in the priority document. Consequently, the relevant date for the subject-matter of claims 11, 12 and 14 is the filing date of the present application, namely March 19th 1999. Consequently, D1, published on April 2nd 1998, is relevant prior art within the meaning of Article 33 and Rule 64.1 PCT for claims 11, 12 and 14 (see the PCT Guidelines, PCT Gazette-Section IV, V-2.4).

ITEM V:

1 Reference is made to the following documents:

D1 WO 98/13694

D2 CHEMICAL ABSTRACTS, vol. 80, no. 11, 18 March 1974 (1974-03-18)
Columbus, Ohio, US; abstract no. 56313y

D3 B. H. TOH ET AL.: 'The 200- and 150-kDa neurofilament proteins react with IgG autoantibodies from patients with kuru, Creutzfeldt-Jakob disease, and other neurologic diseases.' PNAS, vol. 82, May 1985, pages 3485-3489



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : G01N 33/569		A2	(11) International Publication Number: WO 99/47932 (43) International Publication Date: 23 September 1999 (23.09.99)
<p>(21) International Application Number: PCT/GB99/00876</p> <p>(22) International Filing Date: 19 March 1999 (19.03.99)</p> <p>(30) Priority Data: 9805913.2 19 March 1998 (19.03.98) GB</p> <p>(71) Applicant (for all designated States except US): KING'S COLLEGE, UNIVERSITY OF LONDON [GB/GB]; The Strand, London WC2R 2LS (GB).</p> <p>(72) Inventor; and</p> <p>(75) Inventor/Applicant (for US only): EBRINGER, Alan [GB/GB]; 76 Gordon Road, Ealing, London W5 2AR (GB).</p> <p>(74) Agents: POWELL, Stephen, David et al.; Williams, Powell & Associates, 4 St. Paul's Churchyard, London EC4M 8AY (GB).</p>		<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published Without international search report and to be republished upon receipt of that report.</p>	
<p>(54) Title: DIAGNOSIS OF SPONGIFORM OR DE-MYELINATING DISEASE</p> <p>(57) Abstract</p> <p>A method for detecting a de-myelinating disease or spongiform encephalopathy in mammals comprises testing a biological sample obtained from the mammal for IgA antibodies indicative of infection by an <i>Acinetobacter</i> species. The <i>Acinetobacter</i> species is one which presents to the mammal an antigen which exhibits molecular mimicry with the myelin of the mammal e.g. <i>Acinetobacter calcoaceticus</i>. The antibodies tested for are antibodies which bind to an epitope present in or derived from the <i>Acinetobacter</i> species or to a prepared peptide sequence corresponding thereto or to a conformationally similar peptide sequence e.g. the peptide sequence RFSAWGAE or ISRFAWGEV. The method tests for bovine spongiform encephalopathy, multiple sclerosis and Creutzfeldt-Jacob disease in humans. A test kit uses as the test antigen the whole <i>Acinetobacter</i> organism or at least one prepared peptide sequence as described above and a secondary antibody against the human, bovine, or other mammalian IgA.</p>			

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/00876

2 The subject-matter of claim 13 is not clear. The methods of claims 1, 2, 3, 10, 11 and 12 are exclusively directed to the detection of mammalian IgA. Consequently, the features human and bovine lack antecedents in claims 1, 2, 3, 10, 11 and 12, contrary to the requirements of Article 6 PCT.

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EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/00876

D4 R. L. SIDMAN ET AL.: 'Transmissible spongiform encephalopathy in the gray tremor mutant mouse.' PNAS, vol. 82, January 1985, pages 253-257

2 Industrial applicability (Art. 33(4) PCT):

The subject-matter of claims 1-15 is susceptible of industrial application.

3 Novelty and Inventive step (Art. 33(2) and (3) PCT):

3.1 Claims 1-15, in the light of documents D2-D4

D2-D4 do not disclose a method for detecting de-myelinating conditions or spongiform encephalopathy which is based on the detection of anti-Acinetobacter antigen IgA. Claim 1 is consequently novel over D2-D4 (Art. 33(2) PCT). It follows that claims 2-8 and 10-12 meet also the requirements for novelty over D2-D4.

D2-D4 do not either describe the kit of claim 9 (see also Section VIII below) which would appear to be directed to a kit containing (i) a test antigen (whole Acinetobacter or an epitope of Acinetobacter) and (ii) a secondary antibody against human, bovine or other mammalian IgA. The kit of claim 13 (see also Section VIII below) is also novel over D2-D4, because these documents do not disclose a kit comprising (i) peptide sufficiently similar to bind to the anti-Acinetobacter antibodies and (ii) a secondary antibody against human, bovine or other mammalian IgA. It follows that claims 9 and 13-15 meet the requirements for novelty over D2-D4.

Additionally, none of the said document suggests that Acinetobacter could play a role in these neurological disorders (see description of the present application, figures 1-2). Consequently, the subject-matter of claims 1, 9 and 13 cannot be derived in an obvious way from D2-D4 and claims 1-15 meet the requirements for inventiveness over D2-D4 (Art. 33(3) PCT).

3.2 Claims 11, 12 and 14, in the light of document D1

3.2.1 Novelty (Art. 33(2) PCT)

D1 demonstrates that the presence of anti-Acinetobacter calcoaceticus antibodies (IgA+ IgG + IgM) can be correlated with bovine spongiform encephalopathy (BSE), see D1, pages 4 and 5, fig. 1. The myelin-cross reactive epitope of Acinetobacter calcoaceticus is shown in D1, sequence ISRFAWGEV (cf. D1, Table 1, page 2 and claims). Further, the myelin peptide LSRFSWGAE is mentioned in D1. Finally, a kit comprising one of said peptide is disclosed in D1 (see D1, claims 11 and 12).

D1 is not prejudicial to the novelty of claims 11 and 12, since these claims are directed to a method for detecting IgA antibodies indicative of infection by an Acinetobacter species using peptide ISRFAWGEV or RFSAWGAE. The method of D1 aims at detecting the total immunoglobulins against Acinetobacter (see D1, page 5 and claim 1).

The kit of claim 14 is not anticipated by D1 which does not disclose a kit comprising a combination of (i) peptide RFSAWGAE or ISRFAWGEV and (ii) a secondary antibody against the human, bovine or other mammalian IgA. Claim 14 is therefore novel over D1 (see also Section VIII below).

3.2.2 Inventive step (Art. 33(3) PCT)

D1 discloses a method for detecting demyelinating conditions like BSE and kits thereof. The method of D1 is based on the co-detection of IgG, IgA and IgM antibodies directed against Acinetobacter species.

The problem solved by claims 11 and 12 of the present application over D1 can therefore be seen as the provision of a more accurate method for detecting demyelinating conditions (see description of the present application, page 2, lines 7-11)

The solution proposed in claims 11 and 12 consists in the detection of IgA antibodies directed against Acinetobacter species. The selection of IgA can be regarded as a non-obvious and consequently inventive selection procedure over D1 (see PCT Guidelines,

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/00876

PCT Gazette-Section IV, IV-8.8 (c2)). The subject-matter of claims 11 and 12 is therefore inventive over D1. It follows that claim 14 (see also Section VIII below) which is apparently directed to a kit particularly suitable for carrying out the methods of claims 11 and 12 is also considered to be inventive over D1 (Art. 33(3) PCT).

ITEM VI:

Certain published documents (Rule 70.10)

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO 98/13694	02.04.1998	29.09.1997	27.09.1996

This document (D1) may become relevant in the regional phase of the application because it has an earlier priority date than the present application.

ITEM VIII:

- 1 The wording used in claims 9 and 13 is vague and unclear and leaves the reader in doubt as to the content of the kit, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT). The kits of claims 9 and 13 contain a secondary antibody against the human, bovine or other mammalian IgA; however, the wording chosen by the applicant for defining the first component of the kit "in which the test antigen is.....," does not necessarily imply that the test antigen is present in the kit. This wording might also refer to the method according to any of claims 1 to 8, respectively, claim 10, 11 or 12 and therefore, the test antigen would therefore not be present in the kit, but is simply the antigen to be detected in the method. Consequently, the kits of claims 9 and 13 could be limited to a kit including a secondary antibody against the human, bovine or other mammalian IgA. Clearly, a kit including exclusively this latter component could not be considered to be novel. Claims 9 and 13 are consequently unclear, i.e. the intended limitations are not clear from these claims (Art. 6 PCT). The positive statements given above with respect to novelty and inventive step have been given for a kit containing both components, i.e. the test antigen and the secondary antibody.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
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BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

DIAGNOSIS OF SPONGIFORM OR DE-MYELINATING DISEASE

This invention relates to the diagnosis of de-myelinating diseases and spongiform encephalopathies in animals and humans.

In our copending application WO 98/13694 we have disclosed a new diagnostic test for spongiform encephalopathies and other de-myelinating conditions in mammals. The test disclosed in our prior application is based on a model of the genesis of this pathological state which is applicable to the various forms in which it is manifest in humans and animals. In relation to the bovine spongiform disease this model provides an alternative to the current theory based on the formation of prions. Briefly, this new model is based on the phenomenon of molecular mimicry according to which mammals exposed to certain bacteria having peptide sequences which mimic myelin peptides experience an auto-immune reaction. In our prior application we indicated that human de-myelinating diseases were also open to the same explanation according to our new model disclosed therein.

According to the present invention, a method for detecting a de-myelinating disease or spongiform encephalopathy in mammals comprises testing a biological sample obtained from the mammal for IgA antibodies indicative of infection by an *Acinetobacter* species. We believe that infective micro-organisms of these species present to the mammal an antigen which exhibits molecular mimicry with the myelin of the mammal. The phenomenon of molecular mimicry has been explained in our above-mentioned prior

application WO 98/13694, the contents of which are hereby incorporated by reference.

We have now confirmed the presence of elevated levels of certain antibodies in human sera of patients suffering from multiple sclerosis (MS). These are the IgA antibodies to *Acinetobacter* species e.g. *Acinetobacter calcoaceticus*, the same organisms for which antibodies were previously found in BSE sera. Similar results have been obtained for Creutzfeldt-Jakob disease (CJD). Tests for antibodies in sera from patients who had died of CJD also show increased levels, this being especially marked for the IgA antibody sub-class. The same IgA specificity also applies to bovine sera used for the tests described in our above-mentioned copending application.

It is clear that humans suffering from MS and CJD and cows suffering from BSE all have very significantly raised levels of *Acinetobacter calcoaceticus* IgA antibodies in their blood. Tests for such antibodies in sera from living subjects at an early stage make it possible to identify those liable to develop these diseases. The present invention opens up the opportunity of early treatment of these infections e.g. by use of an appropriate antibiotic to prevent further autoimmune attack on the subjects' own myelin.

As also indicated in our application WO 98/13694, *Acinetobacter calcoaceticus* is one species of *Acinetobacter* which provides an antigen which stimulates the formation of antibodies which cross-react with the mammalian myelin. Antibodies have been demonstrated to react with several strains of this species including 17905, AC606, SP13TV, 105/85, and 11171. These strains are in the

Reference Centre for *Acinetobacter* species held by Dr Kevin Towner, Public Health Laboratory, University of Nottingham, U.K.

In carrying out the present invention, the test is for antibodies which bind to an epitope present in or derived from the *Acinetobacter* species. The antigen used in the test may be the whole organism or at least one prepared peptide sequence corresponding to an *Acinetobacter* epitope. Alternatively, peptide sequences may be used which have minor variations in amino-acid sequence from the above-mentioned epitopes or prepared peptides but are conformationally sufficiently similar to them that they also bind to the relevant antibodies. For example, peptides having the sequence RFSAWGAE or ISRFAWGEV may be used.

A test kit for use according to the invention therefore contains at least one test antigen as just indicated. In order to reveal IgA antibodies the kit also contains a secondary antibody against the human, bovine, or other mammalian IgA.

As indicated in WO 98/13694, antibodies are assayed and a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.

In view of the greater specificity of the IgA antibodies in the immune response it may be concluded that the mechanism of infection with *Acinetobacter* is via the mucous membranes of the body, the primary sites being the gut or the nasal passages. Since a further correlation has been observed between MS sufferers and patients with major sinus infections, it is probable that the nasal passages

EXAMPLE

The assay for the above mentioned organisms is described in our co-pending application mentioned above. The improved method used herein is as follows:-

ELISA TEST

- 1) Aliquots of 200 μ l of the diluted suspension of *Acinetobacter calcoaceticus* (NCIMB 10694, Aberdeen) grown in nutrient broth are absorbed onto 96 well flat bottomed rigid polystyrene microtitre plates overnight at 4°C.
- 2) The plates are then washed 3 times with phosphate buffered saline (PBS), 0.1% (v/v) Tween 20.
- 3) Aliquots of 200 μ l of blocking solution (0.2% w/v ovalbumin, 0.1% v/v Tween 200 in PBS is added to each well and incubated for one hour at 37°C.
- 4) The plates are then washed 3 times with PBS.Tween 20.
- 5) Aliquots of 200 μ l serum samples (test or control) diluted 1/200 in PBS. Tween 20 is added and incubated for 2 hours at 37°C.
6. The plates are then washed 3 times with PBS.Tween 20.
- 7) Aliquots of 200 μ l of peroxidase conjugated rabbit anti-human IgA or rabbit anti-cow IgA , diluted 1/4000 (cow) (or 1/500 for human) with PBS.Tween 20 are added and incubated for 2 hours at 37°C.
- 8) The plates are then washed 3 times with PBS.Tween 20.

9) The development of the colorimetric assay takes place at room temperature for 20 minutes, after the addition of 200 μ l per well of 0.5 mg/ml (2,2'-azinobis(3-ethylbenz-thiazoline-6-sulphonic acid) in citrate/phosphate buffer, pH 4.1, containing 0.98 mM hydrogen peroxide.

10) the reaction is then stopped with 100 μ l of 2 mg/ml sodium fluoride and optical densities measured at a wavelength of 630 nm with a micro-ELISA plate reader.

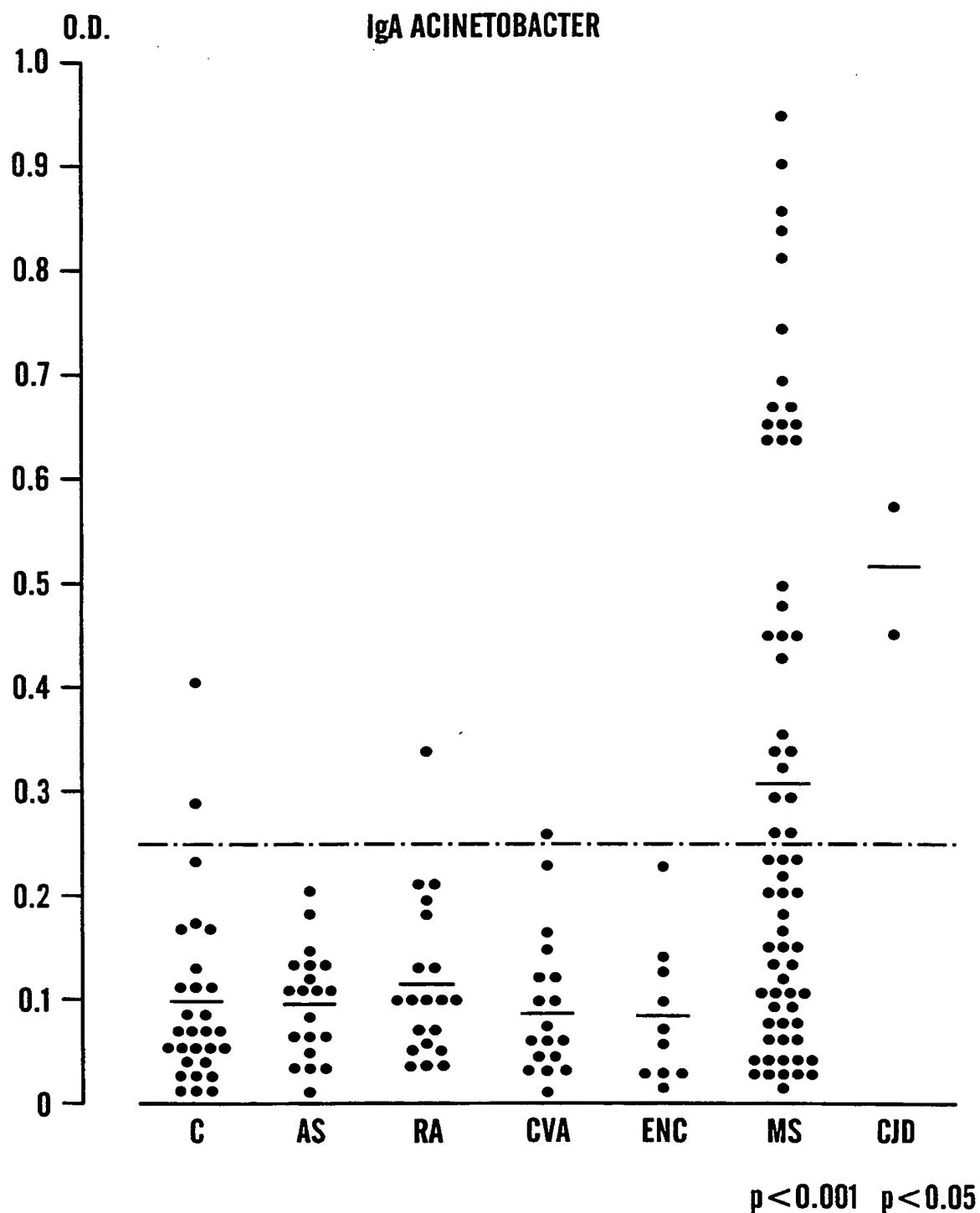
Results for MS and CJD are shown in the attached Figure 1 and those for BSE are shown in Figure 2. These give the titres of IGA *Acinetobacter* antibodies in MS and CJD sera, BSE sera, and control sera. The dashed line represents the 95% confidence limits of the controls.

CLAIMS

1. A method for detecting a de-myelinating disease or spongiform encephalopathy in mammals which comprises testing a biological sample obtained from the mammal for IgA antibodies indicative of infection by an *Acinetobacter* species.
2. A method according to claim 1, in which the *Acinetobacter* species is one which presents to the mammal an antigen which exhibits molecular mimicry with the myelin of the mammal.
3. A method according to claim 1 or 2, in which the antibodies are indicative of prior infection by *Acinetobacter calcoaceticus*.
4. A method according to claim 1, 2, or 3, in which the antibodies tested for are antibodies which bind to an epitope present in or derived from the *Acinetobacter* species or to a prepared peptide sequence corresponding thereto or to a conformationally similar peptide sequence.
5. A method according to claim 4, in which the epitope contains the peptide sequence RFSAWGAE.
6. A method according to claim 4, in which the epitope is the peptide sequence ISRFAWGEV.

7. A method according to any of claims 1 to 6, in which the disease tested for is bovine spongiform encephalopathy.
8. A method according to any of claims 1 to 6, in which the disease tested for is multiple sclerosis in humans.
9. A method according to any of claims 1 to 6, in which the disease tested for is Creutzfeldt-Jacob disease in humans.
10. A method according to any of the preceding claims in which antibodies are assayed and a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.
11. A test kit for use with a method according to any of the preceding claims, in which the test antigen is the whole *Acinetobacter* organism or at least one prepared peptide sequence corresponding to an *Acinetobacter* epitope or a variant peptide sequence which is conformationally sufficiently similar to it to bind to the relevant antibodies, and a secondary antibody against the human, bovine, or other mammalian IgA.
12. A test kit according to claim 11, comprising a peptide having the sequence RFSAWGAE or ISRFAWGEV.
13. A test kit according to claim 11 or 12, in which the secondary antibody is a rabbit anti-human IgA or rabbit anti-bovine IgA.

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LEGEND: IgA ANTIBODIES TO ACINETOBACTER BACTERIA, MEASURED BY ELISA IN HEALTHY CONTROLS (C) AND PATIENTS WITH ANKYLOSING SPONDYLITIS (AS), RHEUMATOID ARTHRITIS (RA), CEREBRO-VASCULAR ACCIDENTS (CVA), VIRAL ENCEPHALITIS (ENC), MULTIPLE SCLEROSIS (MS) AND CREUTZFELDT-JAKOB DISEASE (CJD). (p-VALUES INDICATE SIGNIFICANCE COMPARED TO CONTROLS)

Fig. 1

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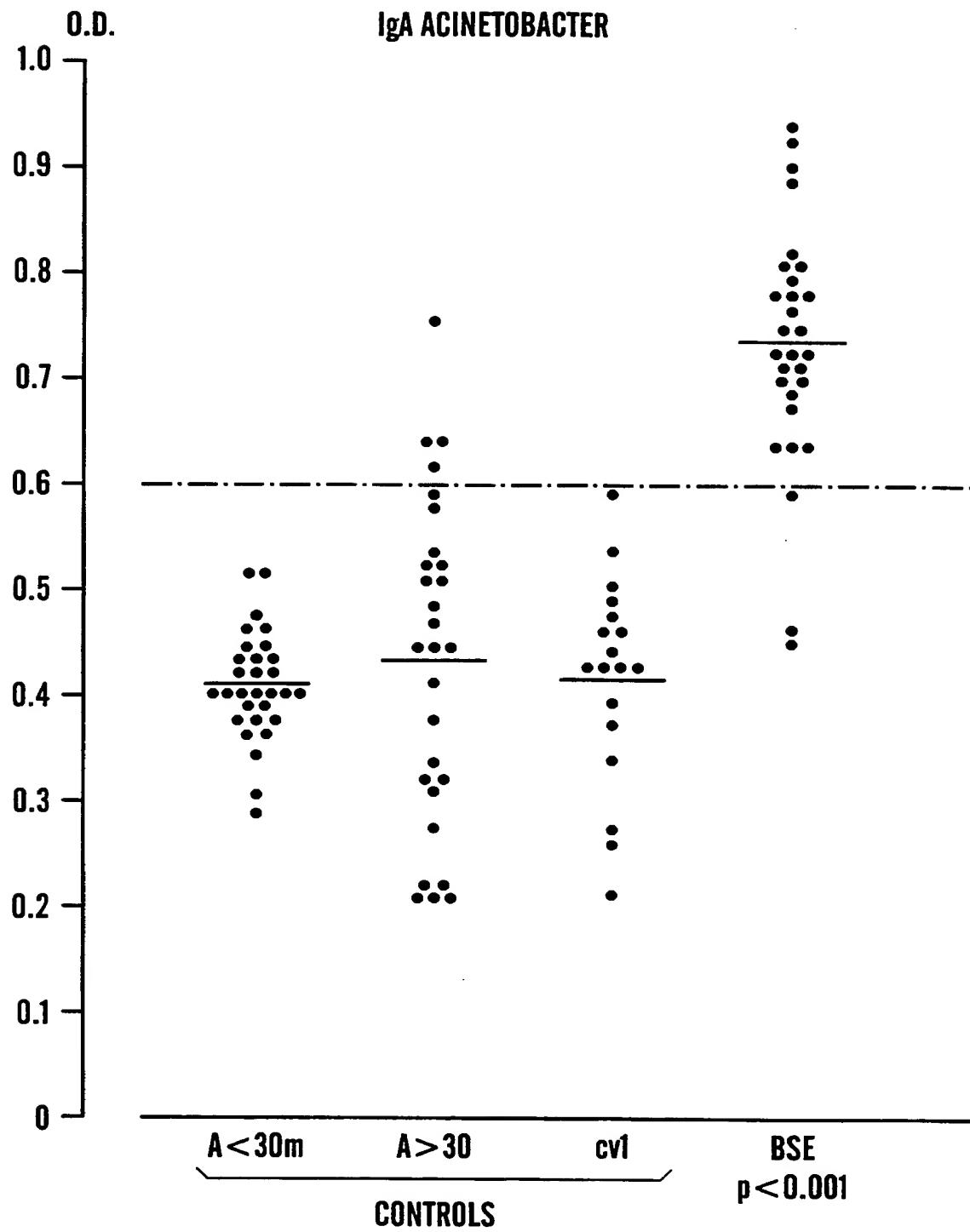


Fig.2



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: PCT/GB99/00876</p> <p>(22) International Filing Date: 19 March 1999 (19.03.99)</p> <p>(30) Priority Data: 9805913.2 19 March 1998 (19.03.98) GB</p> <p>(71) Applicant (for all designated States except US): KING'S COLLEGE, UNIVERSITY OF LONDON [GB/GB]; The Strand, London WC2R 2LS (GB).</p> <p>(72) Inventor; and</p> <p>(75) Inventor/Applicant (for US only): EBRINGER, Alan [GB/GB]; 76 Gordon Road, Ealing, London W5 2AR (GB).</p> <p>(74) Agents: POWELL, Stephen, David et al.; Williams, Powell & Associates, 4 St. Paul's Churchyard, London EC4M 8AY (GB).</p>		<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p> <p>(88) Date of publication of the international search report: 11 November 1999 (11.11.99)</p>	
<p>(54) Title: DIAGNOSIS OF SPONGIFORM OR DE-MYELINATING DISEASE</p> <p>(57) Abstract</p> <p>A method for detecting a de-myelinating disease or spongiform encephalopathy in mammals comprises testing a biological sample obtained from the mammal for IgA antibodies indicative of infection by an <i>Acinetobacter</i> species. The <i>Acinetobacter</i> species is one which presents to the mammal an antigen which exhibits molecular mimicry with the myelin of the mammal e.g. <i>Acinetobacter calcoaceticus</i>. The antibodies tested for are antibodies which bind to an epitope present in or derived from the <i>Acinetobacter</i> species or to a prepared peptide sequence corresponding thereto or to a conformationally similar peptide sequence e.g. the peptide sequence RFSAWGAE or ISRFAWGEV. The method tests for bovine spongiform encephalopathy, multiple sclerosis and Creutzfeldt-Jacob disease in humans. A test kit uses as the test antigen the whole <i>Acinetobacter</i> organism or at least one prepared peptide sequence as described above and a secondary antibody against the human, bovine, or other mammalian IgA.</p>			

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/00876

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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IPC 6 G01N

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>CHEMICAL ABSTRACTS, vol. 80, no. 11, 18 March 1974 (1974-03-18) Columbus, Ohio, US; abstract no. 56313, A. WAJGT.: "Assessment by immunofluorescence methods of humoral antimyelin antibody in rats with cyanide encephalopathy." page 68; column 1; XP002052988 abstract & ANN. IMMUNOL. (POZNAN), vol. 5, no. 1-2, 1973, pages 51-58, ---</p> <p style="text-align: center;">-/-</p>	1

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INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/GB 99/00876

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	B. H. TOH ET AL.: "The 200- and 150-kDa neurofilament proteins react with IgG autoantibodies from patients with kuru, Creutzfeldt-Jakob disease, and other neurologic diseases." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 82, May 1985 (1985-05), pages 3485-3489, XP002052986 WASHINGTON US --- R. L. SIDMAN ET AL.: "Transmissible spongiform encephalopathy in the gray tremor mutant mouse." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 82, January 1985 (1985-01), pages 253-257, XP002052987 WASHINGTON US --- CHEMICAL ABSTRACTS, vol. 109, no. 21, 21 November 1988 (1988-11-21) Columbus, Ohio, US; abstract no. 187890, M. P. MCKINLEY ET AL.: "Developmental regulation of prion protein mRNA in brain." page 484; column 2; XP002052989 abstract & CIBA FOUND. SYMP., vol. 135(Novel Infect. Agents Cent. Nerv. Syst.), 1988, pages 101-116, --- WO 98 13694 A (KING'S COLLEGE) 2 April 1998 (1998-04-02) the whole document -----	
P, X		1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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